

In Vitro Assessment of Equivalence of Occupational Health Risk: Welders

by R. M. Stern*

The possibility of using *in vitro* testing to determine the equivalence of risk for various occupational groups is discussed. In the absence of epidemiological evidence or relevant animal *in vivo* bioassays on which to determine the health effects of specific occupational exposures, it is proposed to use similarities in the *in vitro* response to substances with known (or strongly suspected) and unknown risk to demonstrate their risk equivalence. Identification and evaluation of a high risk "hot spot" due to exposure to Cr(VI) for stainless steel welders is discussed in terms of recent developments in collection, analysis and bioassay of welding fumes.

Introduction

Assessment of health risk due to occupation is difficult in a number of industries where many workers are exposed to wide and varying ranges of potentially toxic substances, especially where delayed health effects such as emphysema and respiratory tract cancer appear only towards or at the end of a long working career, frequently after the critical exposure has ceased. It would be extremely useful to demonstrate that appropriate *in vitro* screening tests could be used in quantitatively assessing health risks associated with some occupational exposures. In the absence of a methodology to estimate risk independently, it would be equally useful to be able to utilize such tests in a semiquantitative fashion to rank in order of biological activity various exposures of alternative technologies. This would permit a reduction of otherwise unquantified risk by replacement of suspected processes with those which were technologically equivalent but which produce exposures of demonstrably reduced biological activity. An alternative approach would be the assessment of equivalent risk whereby exposures in one industry could be demonstrated to produce an *in vitro* response identical or similar to those for comparable substances found in industries where the associated human risk was known. The results of preliminary attempts to use such an approach in the welding industry are described below.

The process of welding, a technology in which 1-2% of the work force is engaged in the industrial-

ized countries, produces fume concentrations of the order of 100-400 mg/m³ in the rising column of heated air directly above the arc. Average breathing zone concentrations depend to a great extent on the technology used, but levels of 5 mg/m³ are currently typical throughout the industry (1, 2).

The extremely wide range of technologies, e.g., manual metal arc (MMA) and metal inert gas (MIG), and their applications to various base materials such as aluminum (AL), mild steel (MS), and stainless steel (SS) creates an extraordinary variety of exposures to over 20 different metals, their alloys and oxides, although a few specific combinations of process and materials do provide several well-defined cohorts with specific process-dependent exposures which lie within relatively narrow ranges (1, 2).

While the presence of high concentrations of certain toxic substances—e.g., NO₂, O₃, Cd—in welding fumes can be related to the risk of acute toxicity in some exposures, the question of whether or not welders suffer an average overincidence of various pneumoconioses (or other delayed health effects) remains unresolved. Wide variations in intergroup incidence, e.g., of small round opacities and of chronic bronchitis, are found in cross-sectional studies (1), and the effects of smoking and population dynamics apparently mask the effect of welding on lung function (3, 4). Over 90 cases of pulmonary fibrosis among welders have been identified (5), although the incidence rate among welders or of the normal population is unknown.

On the other hand, five recent epidemiological studies of large general welding populations (6-10) indicate a statistically significant overincidence of

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respiratory tract cancer (and together show a total of 417 observed compared to 307 expected cases in 420,000 man years). However, the fact that industry-wide exposures are so complex makes the determination of the active carcinogens difficult.

Since exposures within the industry are heterogeneous, attention should be focused on the possibility that there exist "hot spots" of high risk whereby a relatively small cohort of workers contributes disproportionately to the average risk. This may be the case for welders of stainless steels, a stable and identifiable group comprising about 10-15% of the total welding population, who are exposed to fumes containing about 5-10% Cr—30-90% of which is in the form of Cr(VI)—a substance absent in low alloy steels. A typical stainless steel welding cohort has a cumulative exposure to Cr(VI) of 5 mg/m³ (years) (1, 11) comparable to that of workers in the chromate industry (12), where Cr(VI) is a putative carcinogen (13).

The requirements on cohort size, cumulative exposure and latency—which must be satisfied in order to generate an epidemiological study of sufficient power to detect a statistically significant overincidence of respiratory tract cancer—makes studies of stainless steel welders prohibitive in most countries. If it could be demonstrated that occupational exposures of stainless steel welders and of chromate industry workers are biologically equivalent and result in equivalent human risk, then, based on the known risk for the chromate industry, the entire excess respiratory cancer risk for all welders could be assigned to this small subcohort which have been included in the previous studies of the general welding populations.

A preliminary attempt to demonstrate the human risk equivalence of stainless steel welding fumes could be based on comparative studies of the *in vitro* and *in vivo* genotoxicity of welding fumes and various solubility fractions thereof. The *in vivo* transplacental mutagenicity of MMA/SS fumes in the mouse spot test (Fleckentest) has been shown to be equivalent to that of the Cr(VI) content, as compared to that from Na₂Cr₂O₇ (14,15). The quantitative response of sister chromatid exchange (SCE) for mammalian cells in culture to MIG/SS fumes (16,17) has been shown to be due to the water-soluble Cr(VI) content and is equivalent to that of comparable amounts of dichromate; MMA/SS fumes produce a response which is significantly below that expected from their Cr(VI) content. Both serum and water-soluble fractions of Ni, obtained from fume composed of mixtures of Ni and NiO, induce specific (molar) SCE rates in human peripheral lymphocytes comparable with that produced by NiSO₄ (18). This Ni; NiO fume shows, in the BHK cell-transformation

test at equitoxic doses, a transformation potency equal to that of Ni₃S₂ and Ni(CH₃COO)₂ (19). This fume has a tumorigenic potency in rats equal to that of NiS (20).

In order to further develop the methodology of risk equivalence studies, it is necessary to examine some of the details of individual bioassay systems and to insure that collection, analysis and interpretation of the results are appropriate for the actual occupational situations for which comparison is intended. The results of some initial efforts in this direction are described below.

Materials and Methods

Production of Welding Fumes

A welding robot is used to produce a standard reference welding fume under well-controlled, reproducible conditions (21,22). Metal inert gas welding of an 18/8 alloy (Sandvik 3RS17 1.2 mm diameter) wire is performed on matching stainless steel plates under either spray arc (26 V, 285 A) or short arc (17 V, 185 A) conditions: shielding gas flow is 15 L/min argon + 1-2% O₂. A ventilation system provides a flow of 43 L/sec, corresponding to a velocity of 1.5 m/sec in the 0.3 m diameter, 2.0 m long collection column, which is fitted with a cowl at its lower end to insure collection of all fume. An internal disk insures turbulent mixing.

Collection of Welding Fumes

Fumes are collected either by means of a 0.3 m diameter paper filter placed at the top of the column (Whatman #41) (the presence of which reduces the column flow by a factor of 6 with no fume loading), or by means of a special impinger system, followed by a total filter (Millipore 0.2 μ) shown schematically in Figure 1. For convenience, a single impinger can be used, although its efficiency for welding fumes is limited to below approximately 60%. To increase collection efficiency, and to mimic the human respiratory tract, two impingers can be run in series: aggregation of the fume in the 100% humidity of the first impinger results in a conditioning which increases the collection efficiency of the second impinger compared to the first. A parallel filter with the same flow is used to monitor the aerosol passing through the impinger system.

Chemical Analysis of Welding Fumes

Fumes are routinely analyzed for water-soluble and insoluble fractions of Fe, Cr and Cr(VI) (23). It has been shown that for welding fumes, all water-

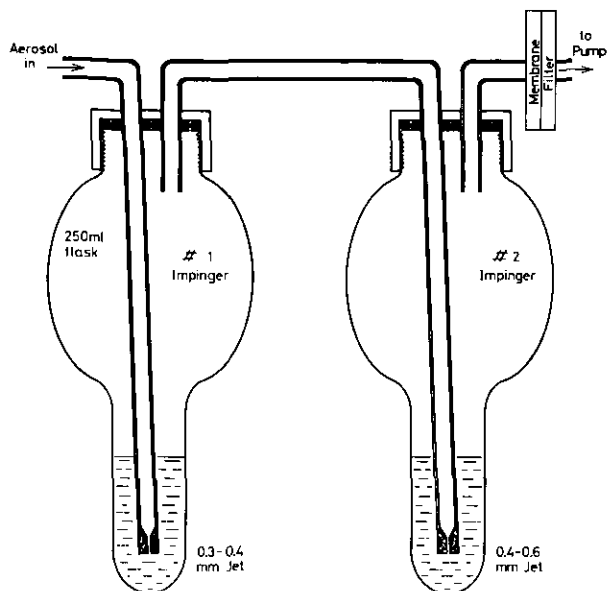


FIGURE 1. Schematic diagram of a two-impinger system for immediate collection of welding fumes. The collection medium determines both the efficiency and the stability of (Cr(VI): doubly deionized, degased water is currently the collection medium of choice. Collection efficiencies can be determined by mass balance or by means of comparison with a parallel filter with similar aerosol flow.

soluble chromium is in the form of Cr(VI), so that only atomic absorption spectrometry is necessary.

Bioassay of Welding Fumes

The mutagenicity of welding fumes was studied using *S. typhimurium* tester strain TA 100 in the histidine revertant plate incorporation test of Ames following standard procedures (24) in the absence of a metabolizing system. In addition, a growing suspension of TA 100 is placed in the (single) impinger system, in order to study the mutagenicity of fresh fume. In this assay, aliquots are removed periodically during collection, half of each to be used for a determination of mutagenicity in minimal agar and half for a measurement of the corresponding survival rate.

Results and Discussion

The results of a large number of studies of the response of TA 100 to various fractions of welding fumes have recently been analyzed (25). The major results are schematically presented in Figure 2, where the number of revertants per plate as a function of calculated Cr(VI) dose, are shown for MMA/SS and MIG/SS welding fumes and for $\text{Na}_2\text{Cr}_2\text{O}_7$. Although the MIG/SS fumes have an extremely wide

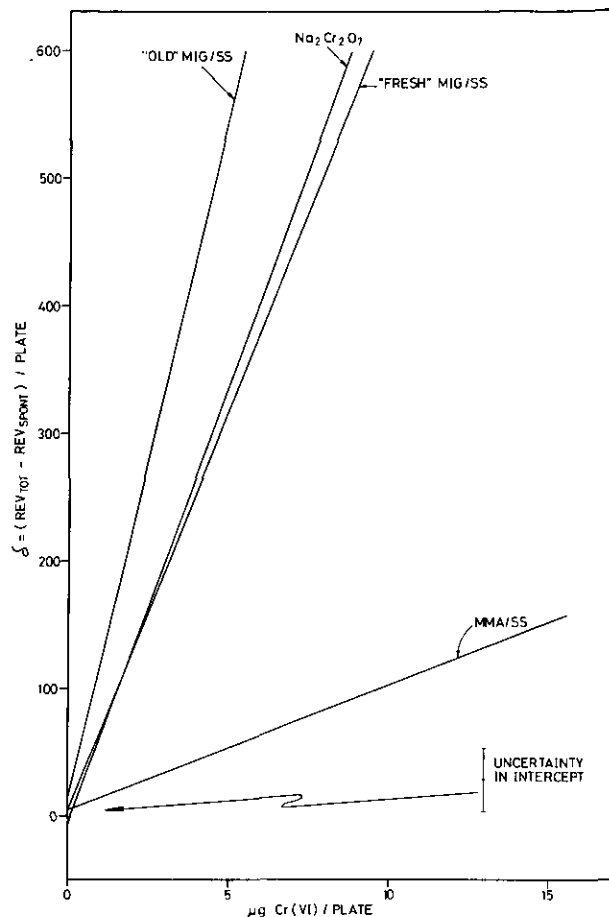


FIGURE 2. Schematic dose-response behavior for TA 100 exposed to MMA/SS, MIG/SS welding fumes and $\text{Na}_2\text{Cr}_2\text{O}_7$. The revertants per plate are plotted as a function of Cr(VI) dose per plate for the various materials.

range of Cr(VI) content (0.02%-0.8%), a linear dose-response relationship is observed when the number of revertants is plotted against the actual Cr(VI) dose per plate. This dose response per mole Cr(VI) is identical with that found for $\text{Na}_2\text{Cr}_2\text{O}_7$, and indicates that Cr(VI) is essentially the only active mutagen in this bioassay. For the MIG/SS fume one can conclude that as far as TA 100 is concerned, Cr(VI) as contained therein has the same mutagenic potency as does Cr(VI) from nonfume sources. The observation of the significant reduction in the specific activity of Cr(VI) as contained in MMA/SS fumes, in agreement with observations of SCE activity in CHO cells (16,17) has not been explained: compared to the MIG/SS fume, the chemistry of the MMA/SS fume matrix is extremely complicated, and the high Ca^{2+} and K^{+} content may well affect the sensitivity of the bioassay system. In an independent determination of the response of TA 100 to Cr(VI) (26), it

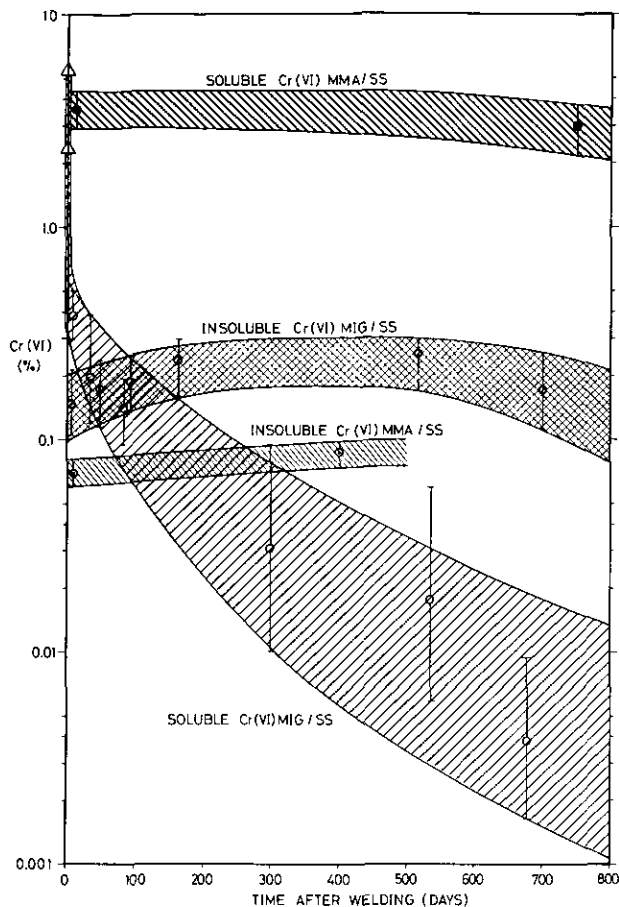


FIGURE 3. Variation of the water-soluble and -insoluble Cr(VI) fractions of MIG/SS and MMA/SS fumes with time after collection and storage at room temperature. The MIG/SS data at "zero" time are from analysis of the impinger system: the rest from fumes collected on paper filters and stored in glass vials.

is observed that although the total-revertant/dose relationships for Cr(VI) from dichromate, MIG/SS and MMA/SS fume are quantitatively the same as reported above, preliminary use of the replica plating technique indicates that all three systems may in fact induce the same number of true revertants per dose Cr(VI): dichromate and MIG/SS fume produce a relative excess of false revertants even at low doses.

The high specific mutagenicity shown for "old" MIG/SS fumes in Figure 2 arises from the fact that chemical analyses for these matrices were performed at a considerable time after the bioassay. It is found that the Cr(VI) content of MIG/SS matrix is not stable, but decays, rapidly at first, and then slowly over long periods of time. Late chemical analysis systematically yielded a Cr(VI) content which was lower than that actually present at the time of the bioassay, hence the apparent increased activity.

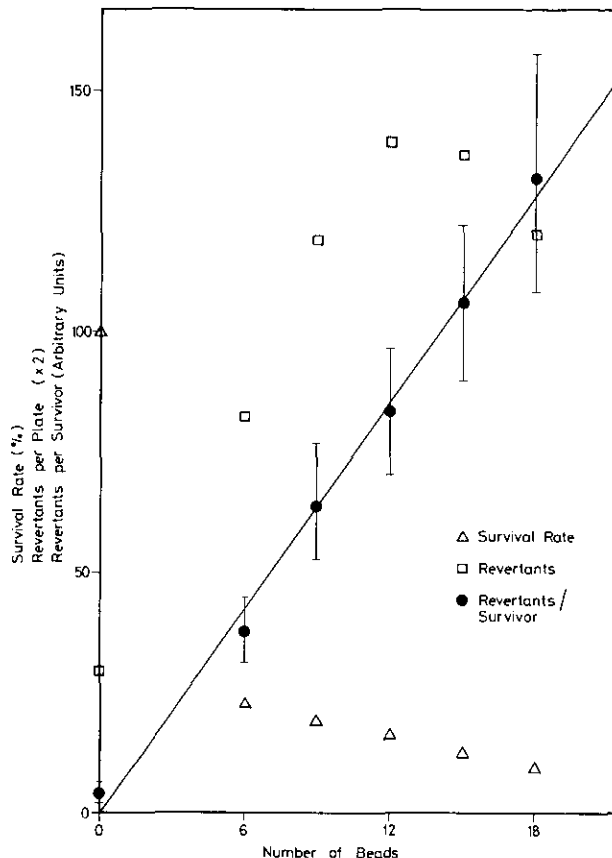


FIGURE 4. Relative number of revertants, fraction of survivors, and relative number of revertants per survivor, as a function of the number of welding beads of MIG/SS, for the impinger bacteria suspension system. The total welding time is 3600 sec, during which 4.8 mg of fume collected in the impinger.

The behavior of Cr(VI) in the various fume matrices with time is shown in Figure 3. Although MMA/SS fume is stable, there is a reduction of Cr(VI) to Cr(III) due to the presence of Fe(II) in the MIG/SS matrix (27). The Cr(VI) content at short times can be established only with the help of the impinger system. Some preliminary results are listed in Table 1. It can be seen that up to 25% of the total Cr is in the form of Cr(VI) shortly after welding. Reduction in Cr(VI) content can also be followed as the fume rises in the collection manifold by withdrawing the aerosol at different points.

Since it is apparent that welding fume contains a short-lived mutagen, it is of interest to develop the *in vitro* bioassay so as to permit a determination of the mutagenic activity of fresh fumes, at a time comparable to that of inhalation. It is found that a growing suspension of TA 100 can be placed in the (single) impinger system, which then serves as a

Table 1. Distribution of chromium in welding fumes.

	Fumes collected in Impingers, % of total ^a												Fumes collected on paper filters, % of total		
	Impinger 1			Impinger 2			Filter			Total			Soluble	Insoluble	Total
	Sol.	Insol.	Total	Sol.	Insol.	Total	Sol.	Insol.	Total	Sol.	Insol.	Total			
MIG/SS ^b															
Cr(VI)	0.144 ^c	0.018	0.162	0.624 ^c	0.018	0.64	0.084 ^c	0.09	0.17	0.852	0.126	0.97	0.005-0.38	0.01-0.54	0.02-0.5
Cr(O + III)	— ^d	1.4	1.4	— ^d	2.48	2.48	— ^d	1.15	1.15	— ^d	5.03	5.03	—	3.56-13.78	3.56-13.78
Total	0.14 ^c	1.42	1.56	0.62 ^c	2.50	3.12	0.084 ^c	1.24	1.32	0.85	5.13	6.00 ^e	0.005-0.38	3.60-14.3	4.06-14.3
MIG/SS ^f															
Cr(VI)	2.10 ^g	—	2.10	—	—	—	0.21	0.14	0.35	2.31	0.14	2.45	—	—	—
Cr(O + III)	0.76 ^g	4.2	4.96	—	—	—	—	3.26	3.26	0.76	7.46	8.22	—	—	—
Total	2.86 ^c	4.2	7.06	—	—	—	0.21	3.40	3.61	3.07	7.60	10.67	—	—	—
MMA/SS ^b															
Cr(VI)	2.11 ^g	—	2.11	1.31	—	1.31	0.15	0.37	0.52	3.57	0.37	3.94	2.2-4.3	0.03-0.42	2.2-4.5
Cr(O + III)	— ^g	0.044	0.044	—	0.044	0.044	—	—	—	—	0.088	0.088	0	0.2-2.1	0.2-2.1
Total	2.11 ^c	0.044	2.15	1.31	0.044	1.35	0.15	0.37	0.52	3.57	0.46	4.02	2.2-4.3	0.2-2.5	2.4-6.4

^aDoubly deionized water; initial pH 7.0, final pH 6.7.^b2.2 L/min; 0.3 mm jet, No. 1 and 2 impinger.^cAAS determination; may contain a significant contribution from suspended Cr (O + III) particles.^dAssumed to be zero.^eMay indicate incomplete recovery.^f6.3 L/min; 0.4 mm jet, No. 1 impinger.^gDPC determination.

useful device for monitoring specific mutagenicity in fumes, from one second onward after welding (28).

Welding can be performed almost continuously with the robot: a series of six beads, each of 180 sec duration, can be run on a single plate, which can be changed in 2-3 min. Figure 4 shows the results of a preliminary impinger run using the same MIG/SS welding wire used in the previous experiments, at a choice of the welding parameters (short arc, 17 V, 185 A) which gives essentially maximum Cr(VI) production (27). Although the number of revertants obtained from each aliquot shows a maximum versus fume dose, the number of survivors falls continuously during the run to an ultimate level of the order of 10% of the initial count, leading to an approximately linear dose-revertants/survivor response.

These studies have reinforced the conclusions that the active mutagen for TA 100 found in welding fumes is Cr(VI) and that this material, at least in the MIG/SS fume matrix, behaves identically as does Cr(VI) from other sources, in this bioassay system. In terms of support for the concept of equivalence of risk, there is evidence that, on an equal dose basis, Cr(VI) in welding fumes has the same biological effect as does Cr(VI) from other sources, and there is no reason to assume that the risk in the welding industry is mitigated, based on the observations to date.

On the other hand the specific activity of Cr(VI) from the MMA/SS matrix is reduced by a factor of 2-4, indicating some interference with the bioassay

due to the presence of the other soluble substances present in the matrix. If the reduced activity could be shown to be due to a true antisnergistic effect, then it might be worthwhile to pursue this line of investigation to effect a simple genotoxic risk reduction.

The fact that Cr(VI) is found to decay rapidly in one fume matrix indicates that there is a serious underreporting of the occupational exposure to this substance under certain conditions [here the presence of a reducing agent, Fe(II)]. Presumably this is true of other mutagens, and any attempt to measure "risk equivalence" by *in vitro* (or *in vivo*) techniques must include the possibility of performing an assay of the actual work place exposures, and not only of grab samples returned to the laboratory for analysis at some later time.

Although the Ames assay with *S. typhimurium* is most probably not appropriate for most metals (29) because of its lack of sensitivity to lipid peroxidation and covalent bonding, it has been convenient for use as a tool to study Cr(VI) and to permit an assessment of the techniques for exposure, collection and analysis of a system about which a great deal is known. The observation of "false" Cr(VI) induced revertants in this system does not affect the conclusions of this article but does restrict the validity of the results to low Cr(VI) doses. The results of other studies in the program of *in vitro* determination of risk equivalence are presented in several concurrent articles (19, 26, 30).

Determination of elemental content in various fractions of welding fumes has been made by E. Thomsen, Technological Institute, Tåstrup. Mutagenicity testing was performed in co-operation with H. Larsen, P. Kiel, and M. Andersen, Royal Danish College of Pharmacy, Copenhagen. This work has been partially supported by The Danish National Fund for Technical-Scientific Research (STVF).

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